

FIG. 1A

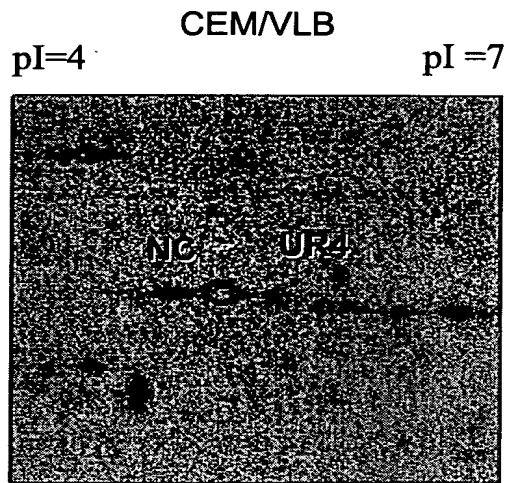


FIG. 1B

Isoform 2 Results

ProFound -Search Result Summary				Version 4.10.8			
© 1997-2000 ProteoMetrics							
Protein Candidates for search 20010608200436-0394208172123151 [73182 sequences searched]							
Rank	Probability	Est'd Z	Protein Information and Sequence Analyse Tools (T)	%	pI	kDa	®
+1	1.0e+000	2.36	gi 5729877 ref NP_006588.1 Heat shock 70 kD protein 8, heat shock 70kD protein 8 (HSP73), heat shock cognate protein, 71 kDa,heat shock 70kd protein 10(HSC71) [Homo sapiens]	25	5.4	71.11	®

FIG. 2A

Search Parameters

Details for rank 1 candidate in search 20010608200436-0394208172123151
gi 5729877 ref NP_006588.1 heat shock 70 kD protein 8, heat shock 70kD protein 8 (HSP73), heat shock cognate protein, 71 kDa,heat shock 70kd protein 10(HSC71) [Homo sapiens]
gi 13639862 ref XP_006086.2 heat shock 70kD protein 8 [Homo sapiens]
gi 123648 sp P11142 HS7C HUMAN HEAT SHOCK COGNATE 71 KDA PROTEIN
gi 87625 pir A27077 dnaK-type molecular chaperone-human
gi 32467 emb CAA68445.1 (Y00371)71 Kd heat shock cognate protein [Homo sapiens]
gi 13273304 gb AAK17898.1 AF352832_1(AF352832) constitutive heat shock protein 70 [Homo sapiens]
Sample ID NO CHANGE [Pass 0]
Measured peptides 20
Matched peptides 12
Min. sequence coverage 25%

FIG. 2B

<u>12 Peptides Sequenced*</u>	Measured Mass (M)	Avg/ Mono	Computed Mass	Error (ppm)	Residues	Missed	Peptide sequence
-1	1250.623	M	1250.611	10	237	246	0 MVNHFIAEFK (1) +O@M;
-2	1252.592	M	1252.608	-13	302	311	0 FEELNADLFR
-3	1406.696	M	1406.712	-12	237	247	1 MVNHFIAEFK (1) +O@M;
-4	1479.743	M	1479.746	-2	300	311	1 ARFEELNADLFR
-5	1486.756	M	1486.693	42	37	49	0 TTPSYVAFTDTER
-6	1690.705	M	1690.718	-7	221	236	0 STAGDTHLGGEDFDNR
-7	1786.968	M	1786.982	-8	172	188	1 IINEPTAAAIAYGLDKK
-8	1820.874	M	1820.883	-5	57	72	1 NQVAMNPTNTVFDAGR (1) +O@M;
-9	1837.001	M	1837.005	-2	326	342	1 LDKSQIHDIIVLVGGSTR
-10	1951.065	M	1951.052	7	452	469	1 DNNLLGKFELTGIPAPR
-11	1980.996	M	1980.990	3	138	155	0 TVTNAVVTVPAYFNDSQR
-12	2773.258	M	2773.318	-21	424	447	0 QTQTFITYSDNQPGVLIQVYEGER

*25% of the amino acids of the HSC70 protein were represented in the amino acids of the mass peptides analyzed.

FIG. 2C

1 MSKGPVAGIDLGTTYSCVGVFQHGKVEIANDQQNRTPSYVAFTDTERLIGDAAKNQVA
61 MNPTNTVFDAAKRLIGRRFDDAVQSDMKHWPFMVNDAGRPKVQVEYKGETKSFYPEEVS
121 SMVLTKMKEIAEAYLGKTVTNAVVTVPAYFNDSQRQATKDAGTIAGLNLRIINEPTAAA
181 IAYGLDKKVGAEARNVLI FDLGGGTFDVSILTIEDGIFEVKSTAGDTHLGGEDFDNRMVNH
241 FIAEFKRKHKKDISENKRAVRRRLRTACERAKRTLSSSTQASIEIDSLYEGIDFYTSITRA
301 RFEELNADLFRGTLDPVEKALRDAKLDKSOIHDIVLVGGSTRIPKIQIKLLQDFFNGKELN
361 KSINPDEAVAYGAAVQAAILSGDKSENVQDLLLLLDVTPLSLGIETAGGVMTVLIKRNNTTI
421 PTKQTQTFTTYSDNQPGVLIQVYEGERAMTKDNLLGKFELTGIPPAPRGVPPQIEVTFDI
481 DANGILNVSVDKSTGKENKITITNDKGRLSKEDIERMVQEAKEYKAEDEKQRDKVSSKN
541 SLESYAFNMKATVEDEKLGKINDEKQKILDKCNEIINWLDKNQTAKEKEEFHQQKELE
601 KVCNPIITKLYQSAGGMPGGMPGGFPPGGGAPPSSGGASSGPTIEEVD

BOLD = Peptides covered by MS MALDI analysis

FIG. 3

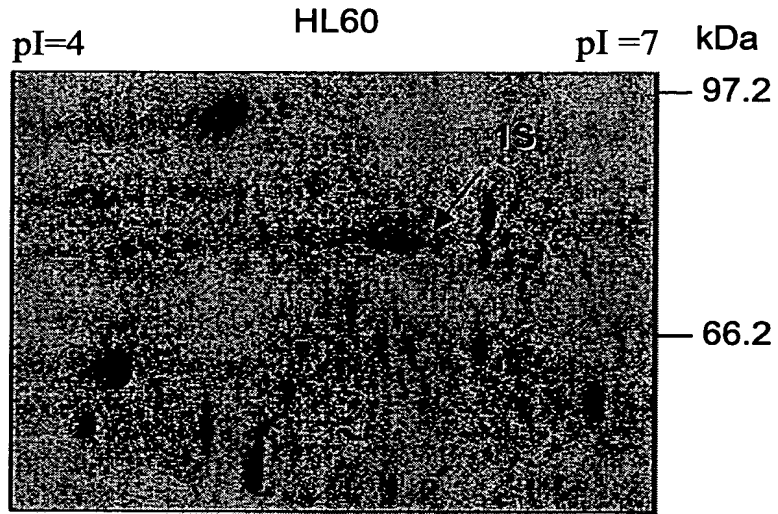


FIG. 4A

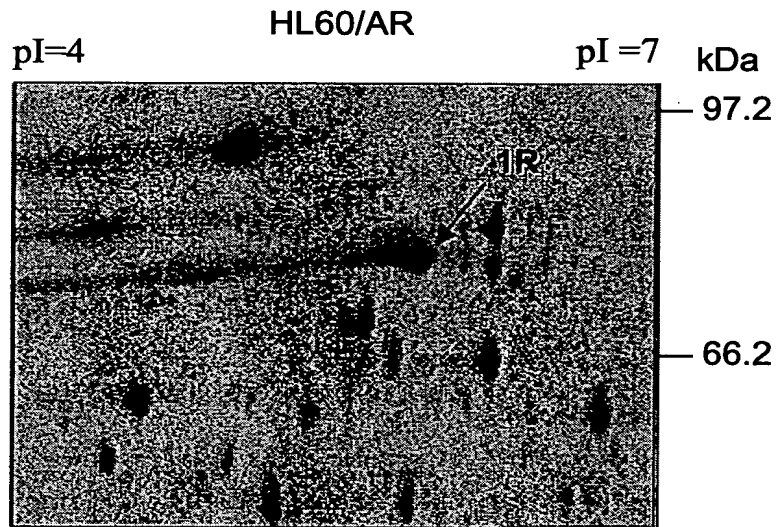


FIG. 4B

ProFound -Search Result Summary		Version 4.10.5 The Rockefeller University Edition				
Protein Candidates for search BD36B5S9-CG5C-289FB336 [88967 sequences searched]						
Rank	Probability	Est'd Z	Protein Information and Sequence Analyse Tools (T)	%	pI	kDa [®]
+1	1.0e+000	2.43	T gi 5729877 ref NP_006588.1 (NM 006597) heat shock 70kd protein 8, heat shock 70kD protein 8(HSP73), heat shock cognate protein 71-kDa, heat shock 70kd protein 10 (HSC71) [Homo sapiens]	<u>26</u>	5.4	71.11 [®]

FIG. 5A

Search Parameters

Input Summary	
Date & Time	Mon Dec 17 01 01:33 2001 UTC (Search Time: 2 80 sec)
Sample ID	
Database	NCBIInr (2001/12/11)
Taxonomy Category	Homo sapiens (human)
Protein Mass Range	60 - 90 kDa
Protein pI range	4.0 - 5.5
Search for	Single protein only
Digest Chemistry	Trypsin
Max Missed Cut	2
Modifications	+C2H3ON@C(Complete), +O@M(Partial), C22H37N4O4S@K(Partial);
Charge State	MH+
Peptide Masses (Da,Average)	
Tolerance(AVG)	100.00 ppm
Peptide Masses (Da,Monoisotopic)	1199.667 1253.610 1401.805 1407.785 1463.128 1480.751 1487.698 1691.729 1830.802 1838.043 1966.104 1982.001 1994.000 2231.141 2275.180 2278.081 2757.251 2774.246 2998.417 3548.224
Tolerance(MON)	100.00 ppm
Number of Peptides	20

FIG. 5B

12 Peptides
Sequenced*

Measured peptides	: 20
Matched peptides	: 12
Min. sequence coverage	: 26%

Boxed peptides are biotinylated

-1	1198.659	M	1198.666	-6	160	171	0	DAGTIAGLNVL
-2	1252.602	M	1252.608	-5	302	311	0	FEELNADLFR
-3	1406.777	M	1406.712	46	237	247	1	MVNRFIAEFKR
-4	1479.744	M	1479.746	-2	300	311	1	ARFEEINADLFR
-5	1486.690	M	1486.693	-2	37	49	0	TTPSYVAFTDTER
-6	1690.721	M	1690.726	2	221	236	0	STAGDTHLGGEDFDNR
-7	1837.035	M	1837.005	16	326	342	1	LDKSQTHDIVLVGGSTR
-8	1965.096	M	1965.083	7	312	325	2	GTLDPVEKALRDAK
-9	1980.994	M	1980.990	2	138	155	0	TVTNAVVTVPAYFNDSOR
-10	1980.994	M	1980.956	19	518	533	3	MVQFAEKYKAEDEKQR
-11	2274.272	M	2274.136	16	57	72	1	NQVAAMNPTNTVFDAGR
-12	2773.238	M	2773.318	-29	424	447	0	QTQFTTYSNQPGLIQVYEGER
-13	2997.409	M	2997.452	-14	540	557	1	NSLESYAFNMKATVEDEK

*26% of the amino acids of the HSC70 protein were represented in the amino acids of the mass peptides analyzed. Note that 2 and 4 represent the same peptide

FIG. 5C

1 MSKGPVAVGIDLGTTYSCVGVFQHGKVEIIANDQGNRTTPSYVAFTDTERLIGDAAK**NQVA**
 61 **MNP****TNTV****FD****AK****R**LIGRRFFDDAVQSDMKHWPFFMVNDAGRPKVQVEYKGETKSFYPEEVS
 121 SMVLT**KM****KE****IA****E**AYLGK**TV****NA****V****TV****P****AY****F****N****S****Q****R****Q****A****T****K****D****A****G****T****I****A****G****L****N****V****L****R****I****I****N****E****P****T****A****A**
 181 IAYGLDKK**V****G****A****E****R****N****V****L****F****D****L****G****G****T****F****D****V****S****I****L****T****I****E****D****G****I****F****E****V****K****S****T****A****G****D****T****H****L****G****G****E****D****F****D****N****R****M****V****N****H**
 241 **F****I****A****E****F****K****R****K****H****K****K****D****I****S****E****N****K****R****A****V****R****R****L****R****T****A****C****E****R****A****K****R****T****L****S****S****T****Q****A****S****I****E****I****D****S****L****Y****E****G****I****D****F****Y****T****S****I****T****R****A**
 301 **R****F****E****E****L****N****A****D****L****F****R****G****T****L****D****P****V****E****K****A****L****R****D****A****K****L****D****K****S****I****H****D****I****V****L****V****G****G****S****T****R****I****P****K****I****Q****I****K****L****L****Q****D****F****F****N****G****K****E****L****N**
 361 **K****S****I****N****P****D****E****A****V****A****Y****G****A****V****Q****A****I****L****S****G****D****K****S****E****N****V****O****D****L****L****L****L****D****V****T****P****L****S****L****G****I****E****T****A****G****G****V****M****T****V****L****I****K****R****N****T****T****I**
 421 **P****T****K****Q****T****T****T****T****S****D****N****Q****P****G****V****L****I****Q****V****E****G****E****R****A****M****T****K****D****N****N****L****L****G****K****F****E****L****T****G****I****P****P****A****P****R****G****V****P****Q****I****E****V****T****F****D****I**
 481 **D****A****N****G****I****L****N****V****S****A****V****D****K****S****T****G****K****E****N****K****I****T****T****I****N****D****K****R****L****S****K****E****D****I****E****R****M****V****Q****E****A****E****K****Y****K****A****E****D****E****K****Q****R****D****K****V****S****S****K****N**
 541 **S****L****E****S****Y****A****F****N****M****K****A****T****V****E****D****E****K****L****Q****G****K****I****N****D****E****D****K****Q****K****I****L****D****K****N****E****I****N****W****L****D****K****N****Q****T****A****E****K****E****E****F****E****H****Q****Q****K****E****L****E**
 601 **K****V****C****N****P****I****I****T****K****L****Y****Q****S****A****G****G****M****P****G****G****F****P****G****G****G****A****P****P****S****G****G****A****S****S****G****P****T****I****E****E****V****D**

BOLD = Nonbiotinylated peptides specific for HSC70
Underlined & Italics = Biotinylated peptides

FIG. 6

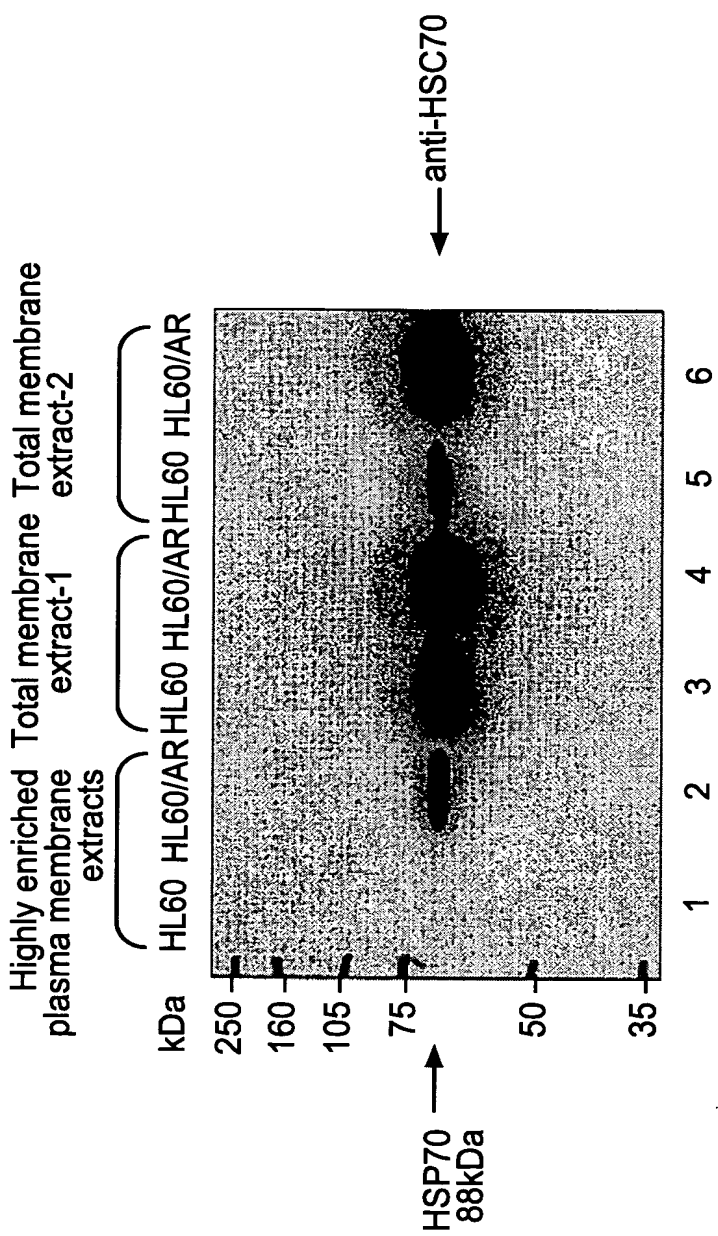


FIG. 7

Biotinylated total cell extracts

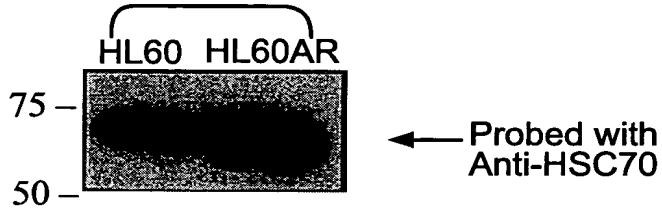


FIG. 8A

Streptavidin purified extracts

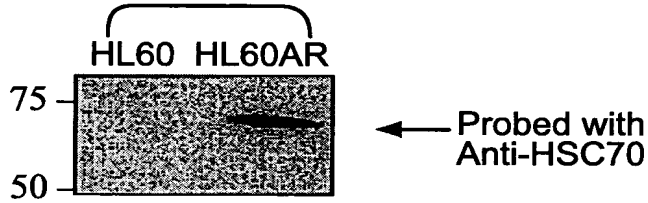


FIG. 8B

IP with anti-HSC70

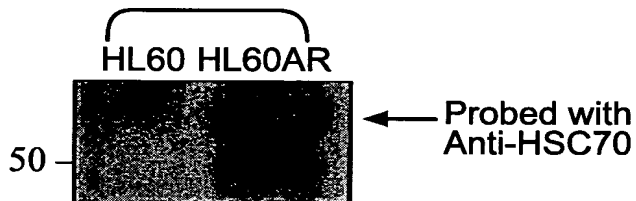


FIG. 8C

IP with anti-HSC70

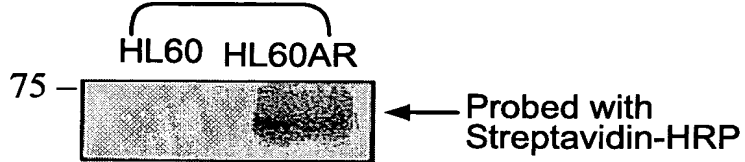


FIG. 8D

Biotinylated total cell extracts

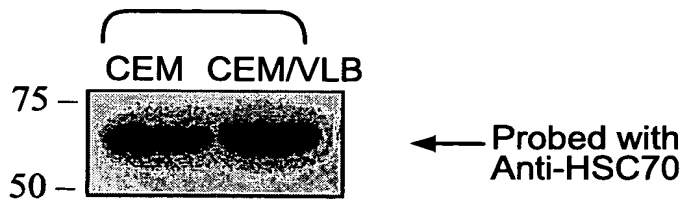


FIG. 9A

Streptavidin purified extracts

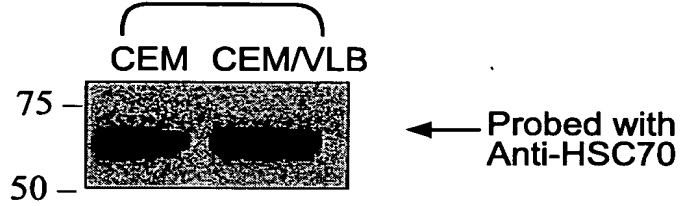


FIG. 9B

IP with anti-HSC70

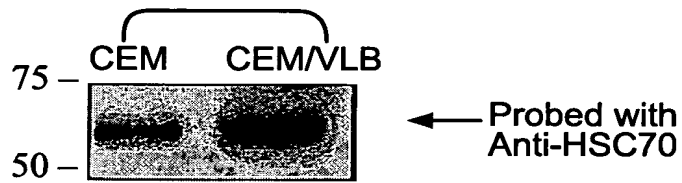


FIG. 9C

IP with anti-HSC70

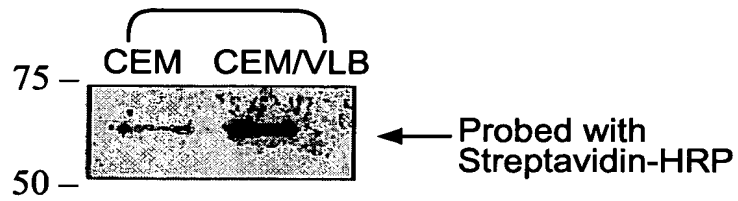


FIG. 9D

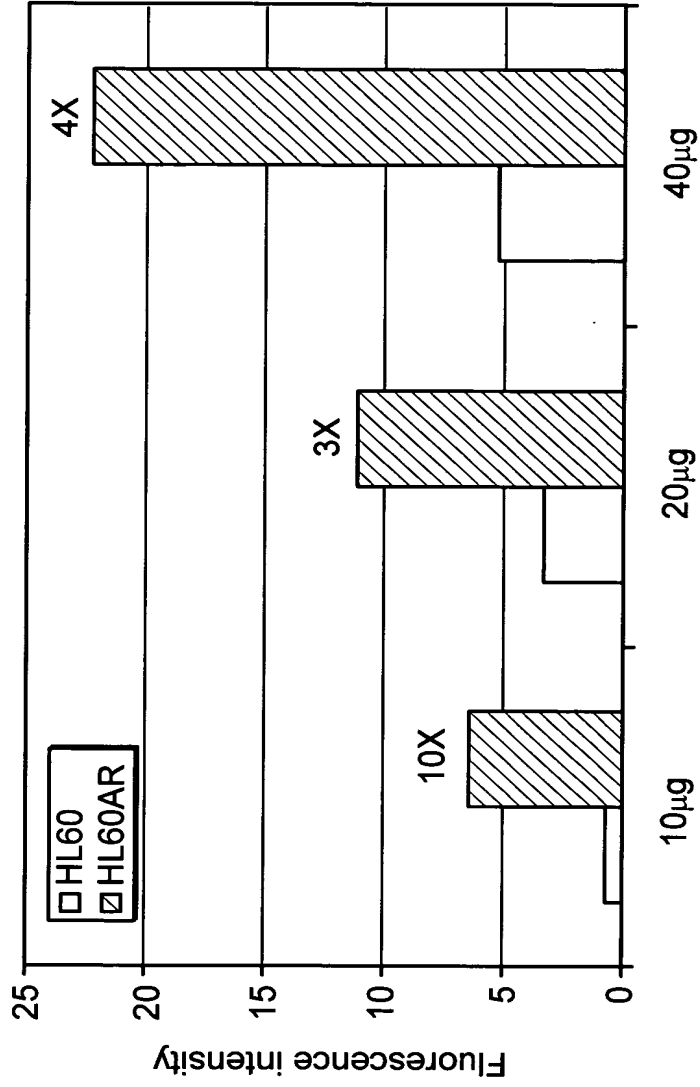


FIG. 10A

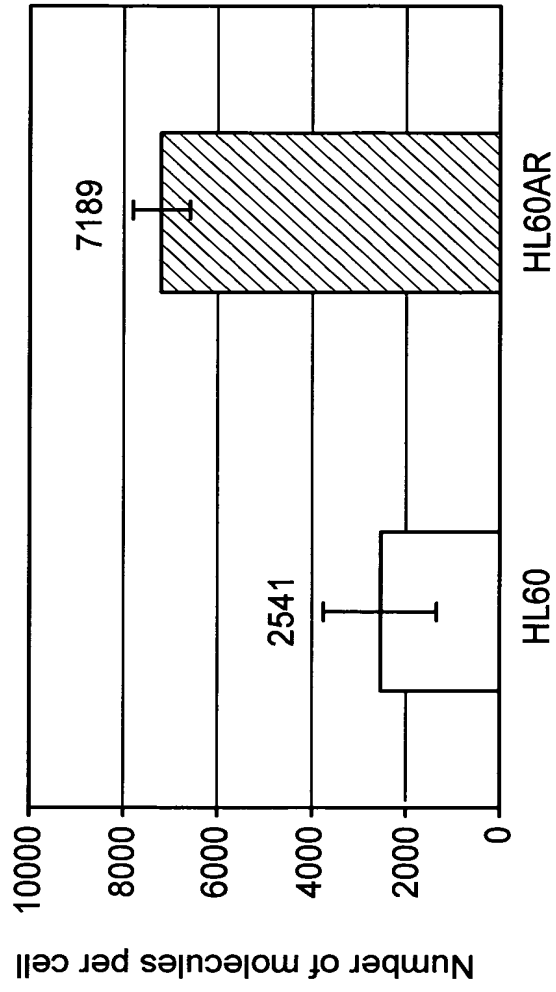


FIG. 10B

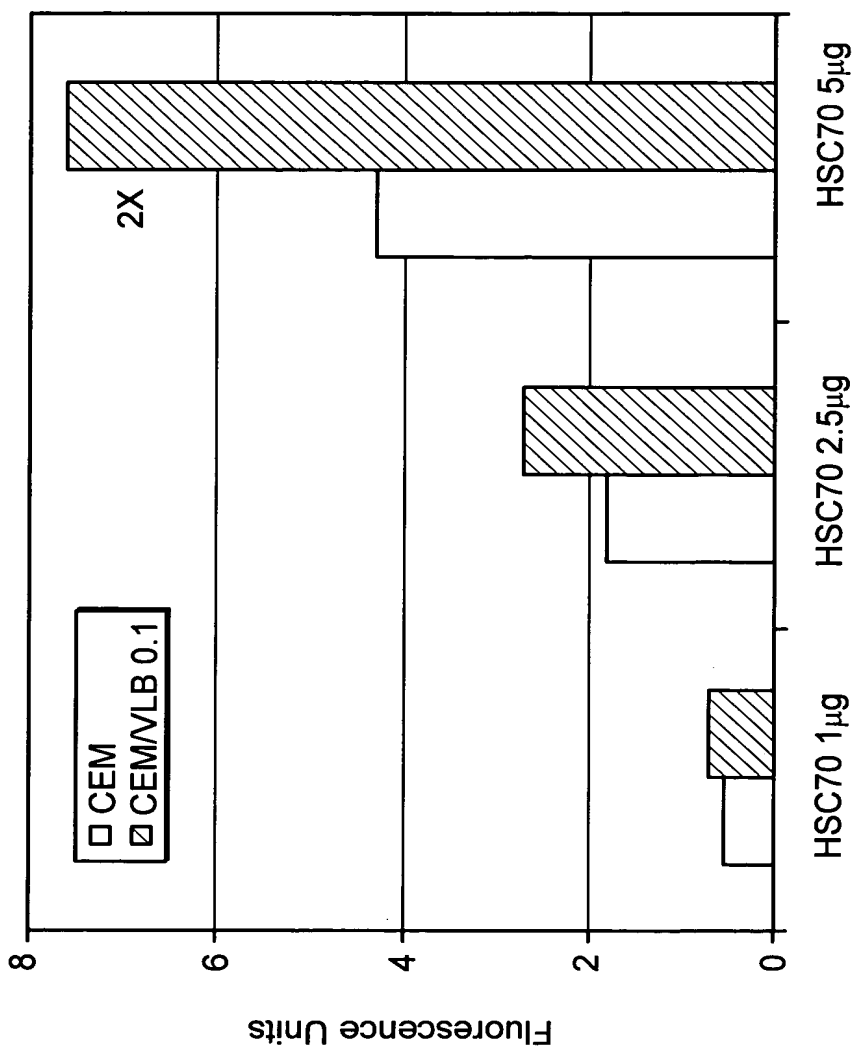


FIG. 11A

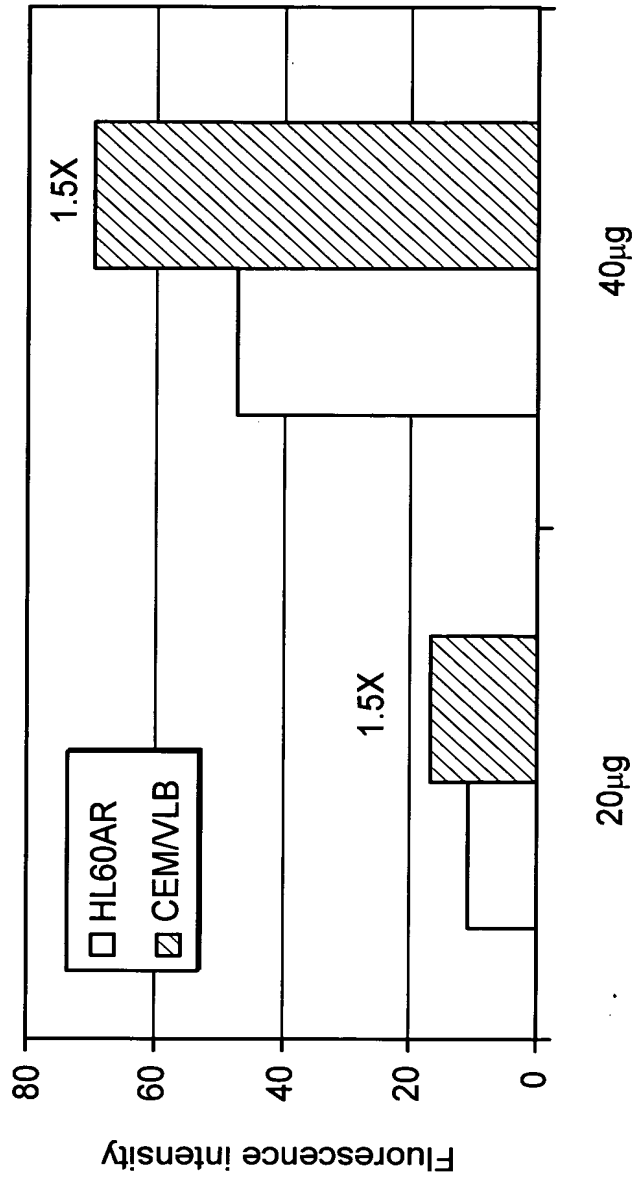


FIG. 11B

17/28

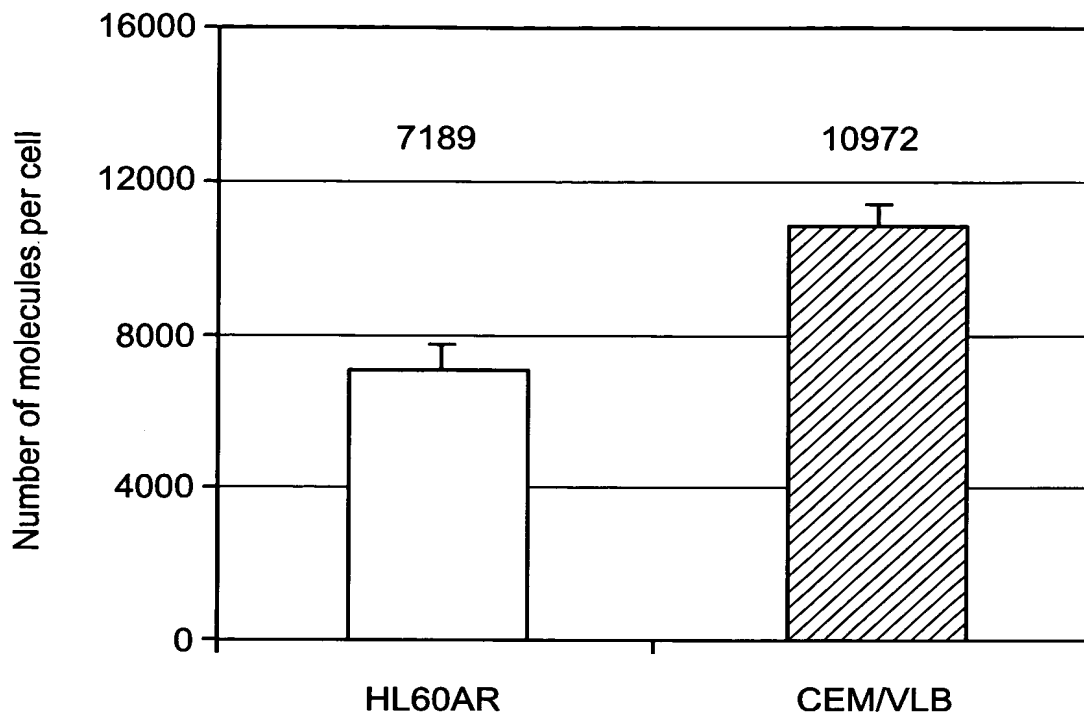


FIG. 11C

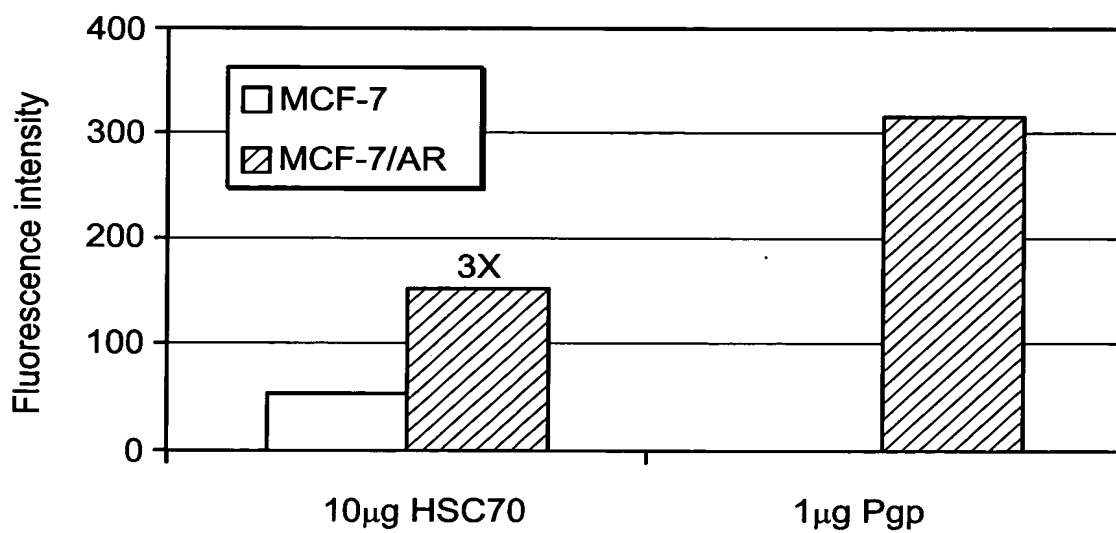


FIG. 12A

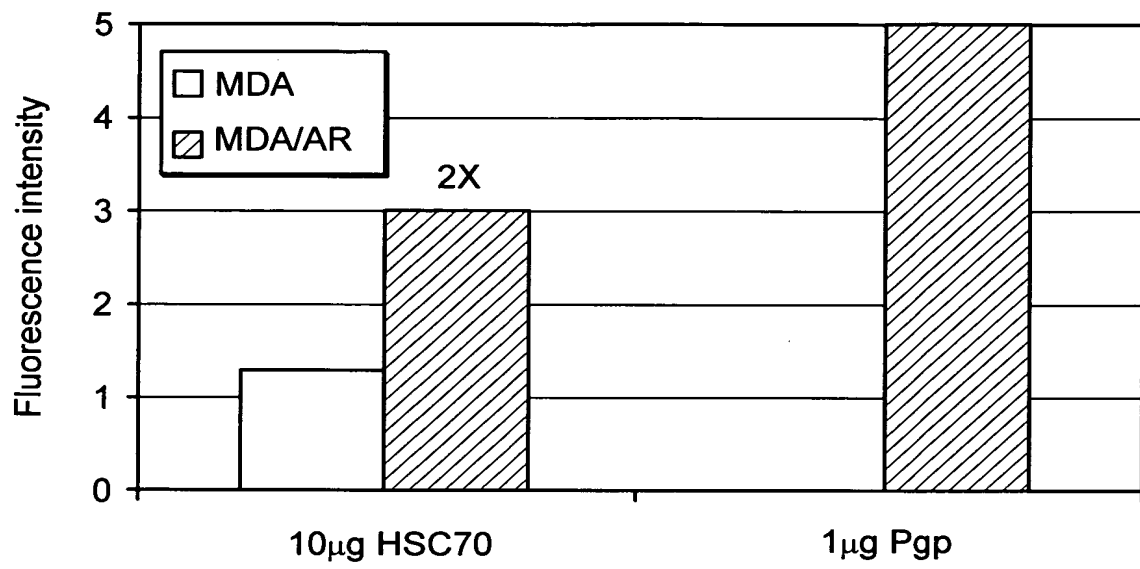


FIG. 12B

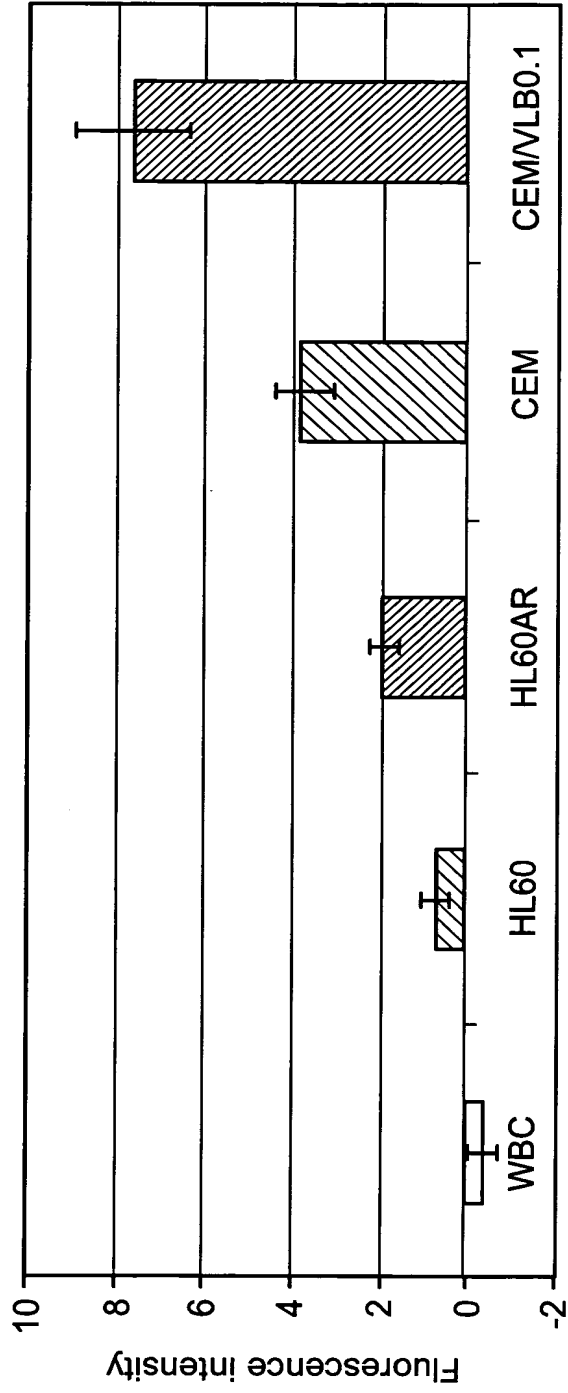


FIG. 13A

**POLYPEPTIDE SEQUENCE OF HUMAN HSC70
(GENBANK ACCESSION NO. AAK17898 (SEQ ID NO. 1))**

1 MSKGPAVGID LGTTYSCVGV FQHGKVEIIA NDQGNRTTPS YVAFTDTERL IGDAAKNQVA
61 MNPTNTVFDA KRLIGRRFDD AVVQSDMKHW PFMVVNDAGR PKVQVEYKGE TKSFYPEEVS
121 SMVLTKMKEI AEAYLGKTVT NAVTVPAYF NDSQRQATKD AGTIAGLNVL RIINEPTAAA
181 IAYGLDKKVG AERNVLIFDL GGGTFDVSIL TIEDGIFEVK STAGDTHLGG EDFDNRMVNH
241 FIAEFKRKHK KDISENKRAV RRLRTACERA KRTLSSSTQA SIEIDSLYEG IDFYTSITRA
301 RFEELNADLF RGTLDPVEKA LRDAKLDKSQ IHDIVLVGGS TRIPKIQKLL QDFFNGKELN
361 KSINPDEAVA YGAAVQAAIL SGDKSENVQD LLLLDVTPLS LGIETAGGVM TVLIKRNTTI
421 PTKQTQFTT YSDNQPGVLI QVYGERAMT KDNLLGKFE LTGIPPAPRG VPQIEVTFDI
481 DANGILNVA VDKSTGKENK ITITNDKGR L SKEDIERMVQ EAEKYKAEDE KORDKVSSKN
541 SLESYAFNMK ATVEDEKLQK KINDEDKQKI LDKCNEIINW LDKNQTAEKE EFEHQQKELE
601 KVCNPIITKL YQSAGMPGG MPGGFPGGGA PPSGASSGP TIEEVD

FIG. 14A

FIG. 14B-1
FIG. 14B-2
FIG. 14B-3

FIG. 14B

**DNA SEQUENCE OF HUMAN HUMAN HSC70
(GENBANK ACCESSION NO. AF352832 (SEQ ID NO. 2))**

1 ATGTCCAAGG GACCTGCAGT TGGTATTGAT CTTGGCACCA CCTACTCTTG TGTGGGTGTT
61 TTCCAGCAGG GAAAAGTCGA GATAATTGCC AATGATCAGG GAAACCGAAC CACTCCAAGC
121 TATGTCGCCT TTACGGACAC TGAACGGTTG ATCGGTGATG CCGCAAAGAA TCAAGTTGCA

FIG. 14B-1

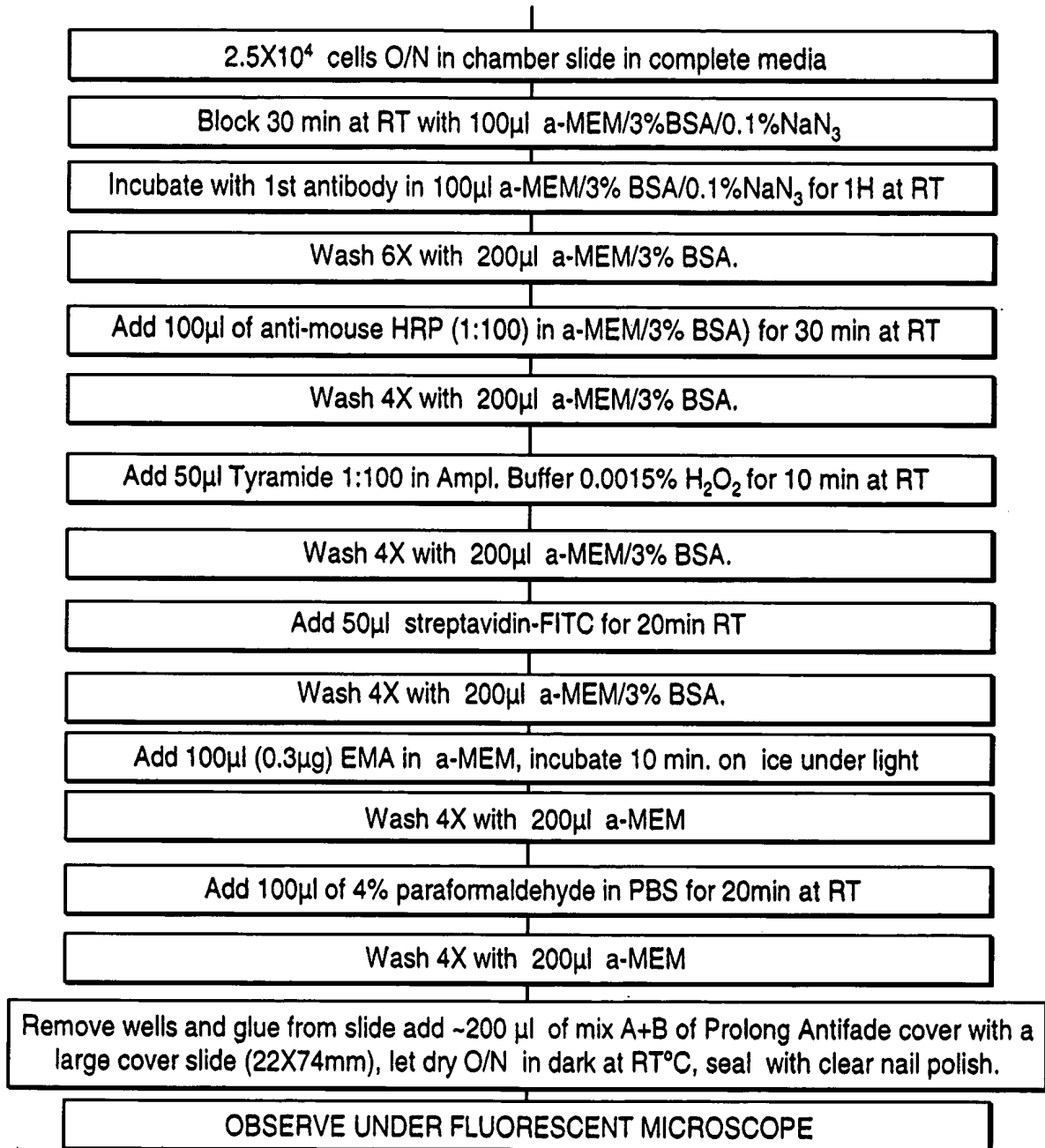
181 ATGAAACCCCA CCAACACAGT TTTTGATGCC AAACGTCTGA TTGGACGCAG ATTTGATGAT
241 GCTGTTGTCC AGTCTGATAT GAAACATTGG CCCTTTATGG TGGTGAATGA TGCTGGCAGG
301 CCCAAGGTCC AAGTAGAATA CAAGGGAGAG ACCAAAAGCT TCTATCCAGA GGAGGTGTCT
361 TCTATGGTTC TGACAAGAT GAAGGAAATT GCAGAAGCCT ACCTTGGGAA GACTGTTACC
421 AATGCTGTGG TCACAGTGCC AGCTTACTTT AATGACTCTC AGCGTCAGGC TACCAAAGAT
481 GCTGGACTA TTGCTGGTCT CAATGTACTT AGAATTATTA ATGAGCCAAC TGCTGCTGCT
541 ATTGCTTACG GCTTAGACAA AAAGGTGGA GCAGAAAGAA ACGTGCTCAT CTTTGACCCTG
601 GGAGGTGGCA CTTTGTGATG GTCAATCCTC ACTATTGAGG ATGGAATCTT TGAGGTCAAG
661 TCTACAGCTG GAGACACCCA CTGGGTGGA GAAGATTTG ACAACC GAAT GGTCAACCAT
721 TTTATTGCTG AGTTAAGCG CAAGCATAAG AAGACATCA GTGAGAACAA GAGAGCTGTA
781 AGACGCCCTC G TACTGCTTG TGAACGTGCT AAGCGTACCC TCTCTTCCAG CACCCAGGCC
841 AGTATTGAGA TCGATTCTCT CTATGAAGGA ATCGACTTCT ATACCTCCAT TACCCGTGCC
901 CGATTTGAAG AACTGAATGC TGACCTGTTC CGTGGCACCC TGGACCCAGT AGAGAAAGCC
961 CTTGAGATG CCAAAC TAGA CAAGTCACAG ATTCATGATA TTGTCCCTGGT TGGTGGTTCT
1021 ACTCGTATCC CCAAGATTCA GAAGCTTCTC CAAGACTTCT TCAATGGGAA AGAACTGAAT

22/28

FIG. 14B-2

1081 AAGAGCATCA ACCCTGATGA AGCTGTTGCT TATGGTGCAG CTGTCCAGGC AGCCATCTTG
1141 TCTGGAGACA AGTCTGAGAA TGTTCAAGAT TTGCTGCTCT TGGATGTCAC TCCTCTTTCC
1201 CTTGGTATTG AACTGCTGG TGGAGTCATG ACTGTCCCTCA TCAAGCGTAA TACCACCATT
1261 CCTACCAAGC AGACACAGAC CTTCACTACC TATTC TGACA ACCAGCCTGG TGTGCTTATT
1321 CAGGTTTATG AAGCGGAGCG TGCCATGACA AAGGATAACA ACCTGCTTGG CAAGTTTGAA
1381 CTCACAGGCA TACCTCCTGC ACCCCGAGGT GTTCCTCAGA TTGAAGTCAC TTTTGACATT
1441 GATGCCAATG GTATACTCAA TGTCTCTGCT GTGACAAGA GTACGGGAAA AGAGAACAAG
1501 ATTACTATCA CTAATGACAA GGGCCGTTTG AGCAAGGAAG ACATTGAACG TATGGTCCAG
1561 GAAGCTGAGA AGTACAAAGC TGAAGATGAG AAGCAGAGGG ACAAGGTGC ATCCAAGAAT
1621 TCACTTGAGT CCTATGCCTT CAACATGAAA GCAACTGTTG AAGATGAGAA ACTTCAAGGC
1681 AAGATTAACG ATGAGGACAA ACAGAAGATT CTGGACAAGT GTAATGAAAT TATCAACTGG
1741 CTTGATAAGA ATCAGACTGC TGAGAAGGAA GAATTTGAAC ATCAACAGAA AGAGCTGGAG
1801 AAAGTTTGA ACCCCATCAT CACCAAGCTG TACCAGAGTG CAGGAGGCAT GCCAGGAGGA
1861 ATGCCCTGGG GATTTCCTGG TGGTGGAGCT CCTCCCTCTG GTGGTGCTTC CTCAGGGCCC
1921 ACCATTGAAG AGGTTGATTA A

FIG. 14B-3

Procedure for immunofluorescence (non-permeabilized cells)

Amplification kit used:
 TSA kit #2 with
 HRP-goat anti-mouse
 IgG and Alexa fluor 488
 tyramide from molecular
 probes T-20192

FIG. 15A

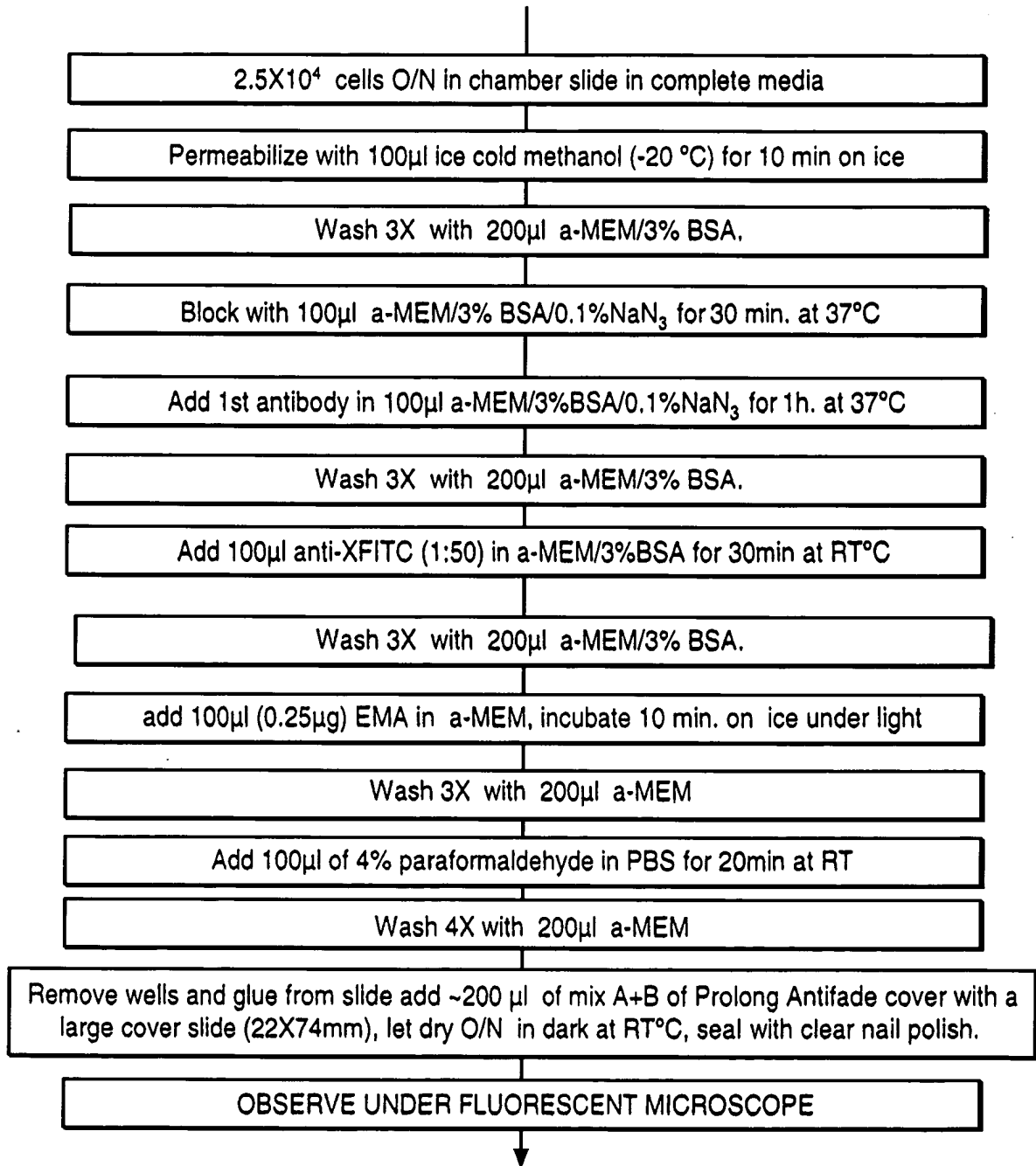
Procedure for immunofluorescence (permeabilized cells)

FIG. 15B

Immunofluorescence of surface exposed HSC70

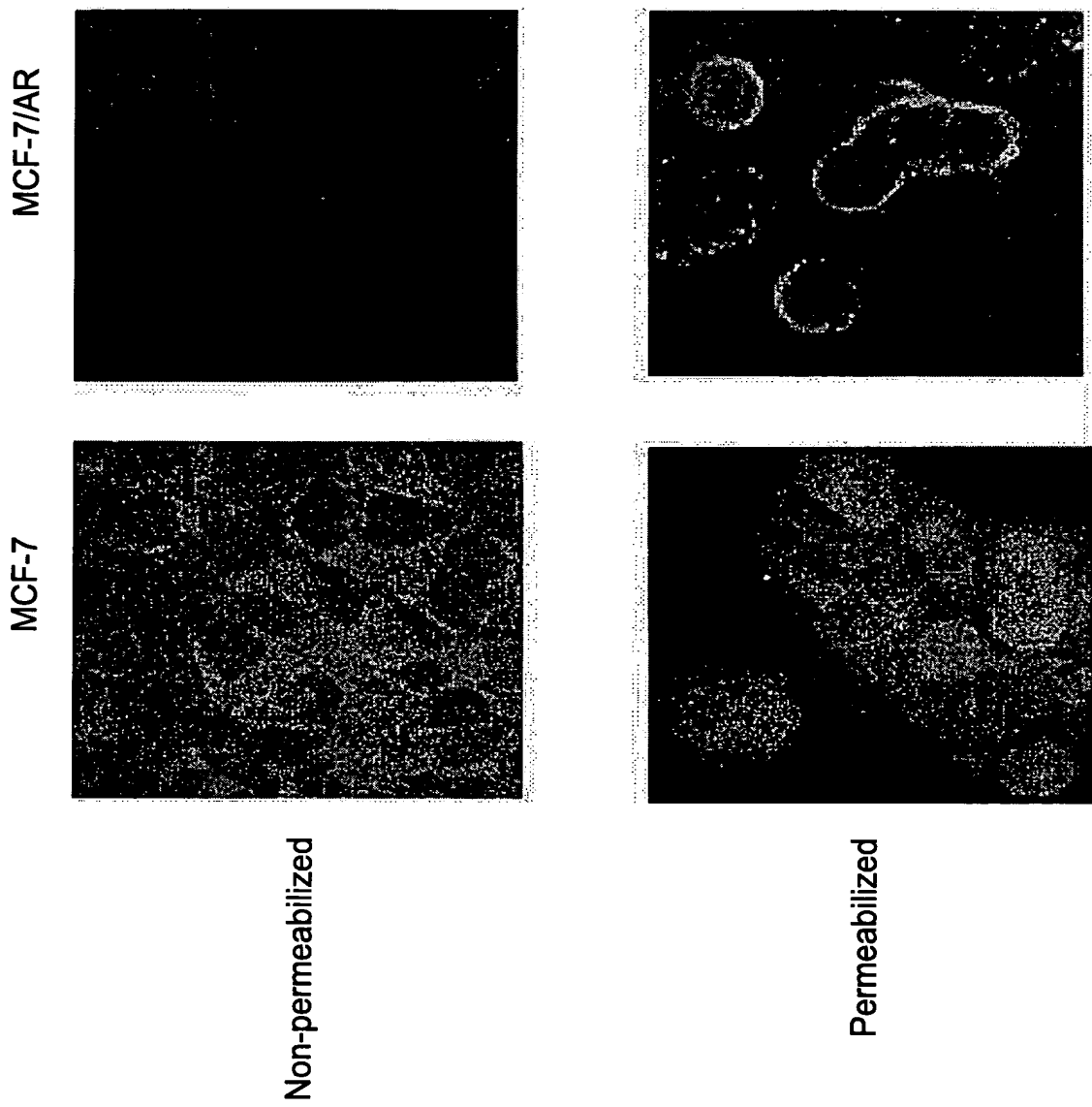


FIG. 16

HSC70 expression

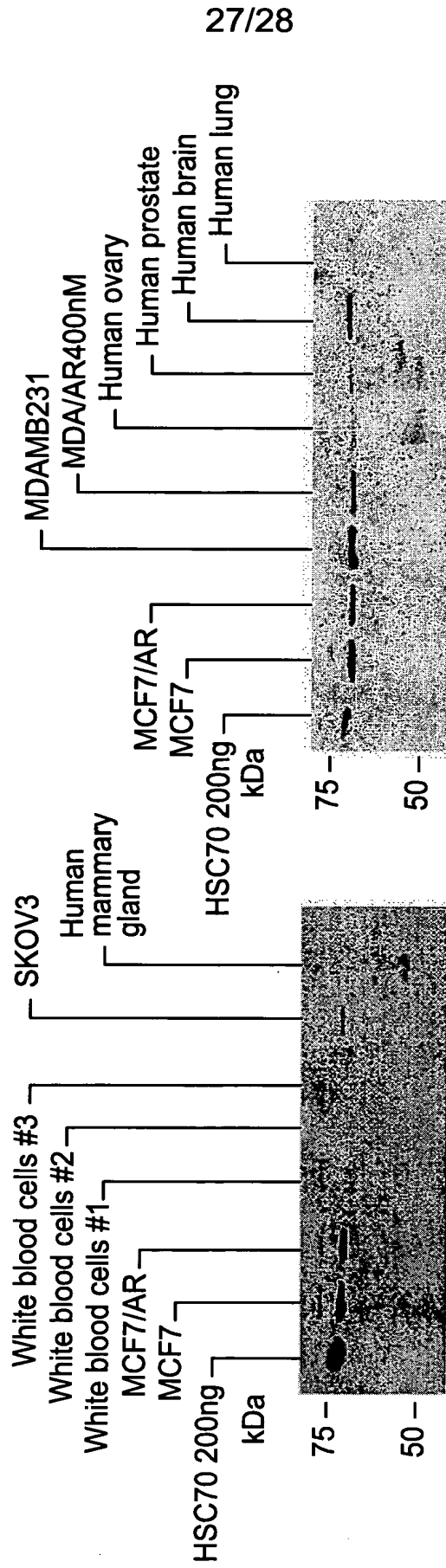


FIG. 17

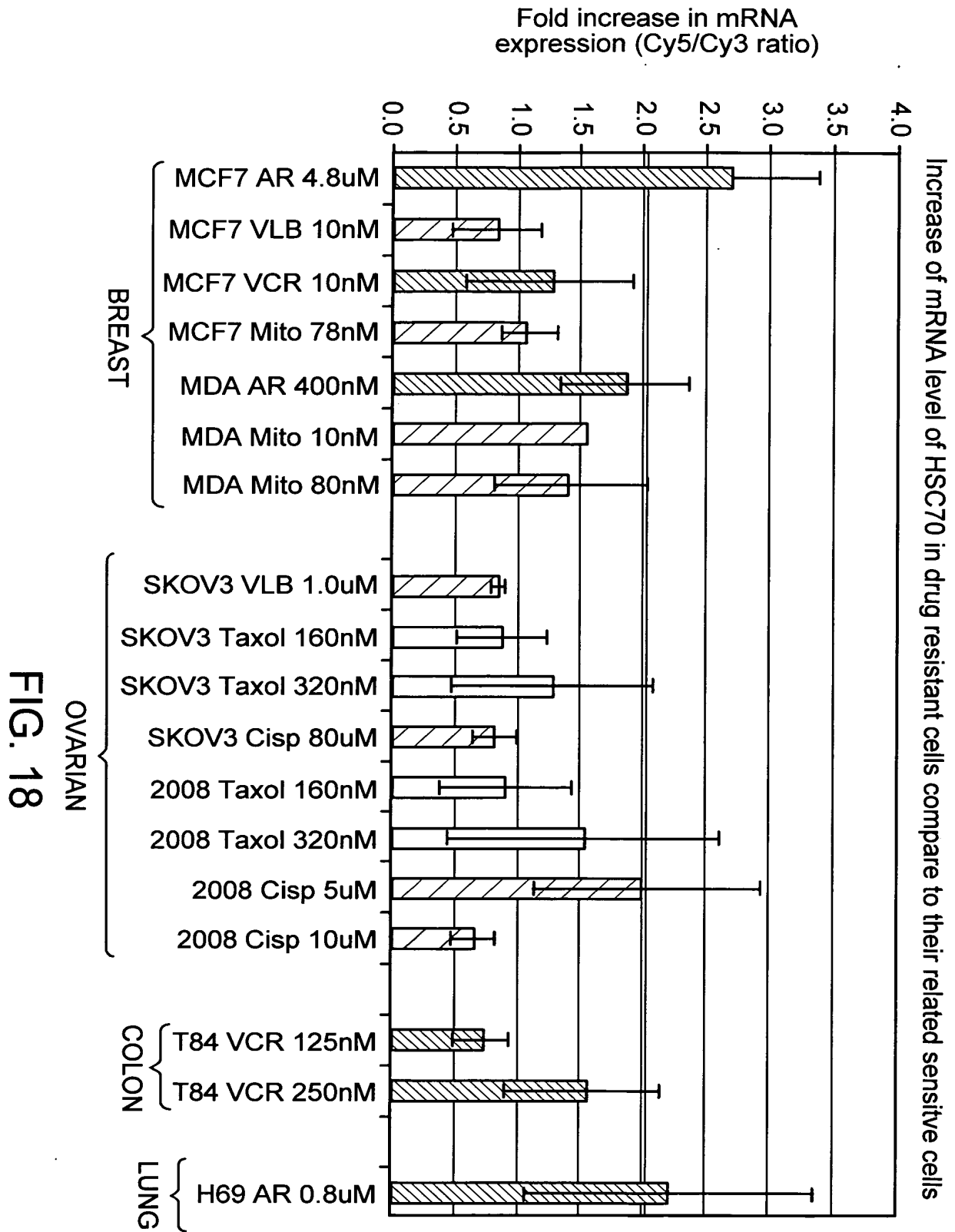


FIG. 18